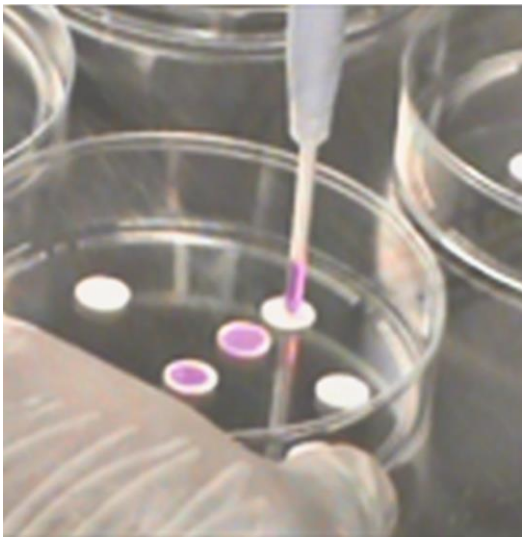
LENA BIOSCIENCES

Innovative 3D Cell Culture Tools for Life Sciences

SeedEZ INSTRUCTIONS



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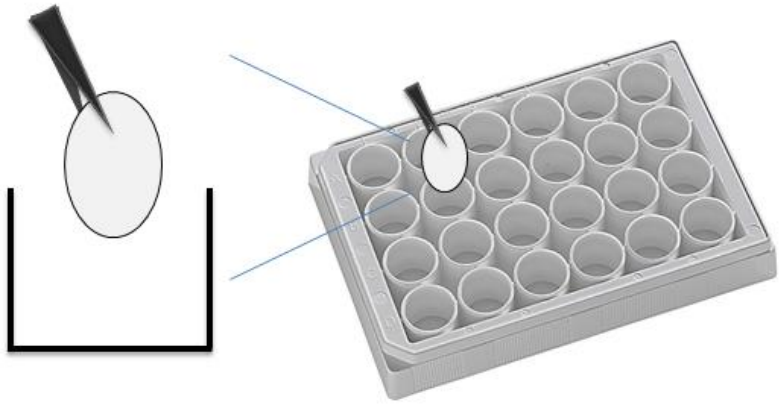
NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES. NOT FOR USE IN ANIMALS OR HUMANS.

INSTRUCTIONS

Step 1: Place the product in a sterile cell culture hood and open the package.

Step 2: Place SeedEZ scaffolds into a multiwell plate

1. Pick up one SeedEZ scaffold at a time using sterile forceps.
2. Place each SeedEZ scaffolds into a multiwell plate (one scaffold per well).



Step 3: If you do not pre-coat multiwell plates for planar cell culture

- Skip this step.

If you pre-coat multiwell plates for planar (2D) cell culture

- Use the same protocol and deliver coating solution into the scaffold.

Note:

If the cells adhere poorly to plastic disposables in 2D, then they will adhere poorly to SeedEZ. If you do not culture spheroids, use 100 µg/ ml Poly-D-Lysine solution to pre-coat SeedEZ.

Step 4: Pre-wet SeedEZ and aspirate the same volume prior to addition of cells.

A. Add DI water to SeedEZ and let it spread (the scaffold appears transparent):
   - SeedEZ SC-C006-0006: add 450 µl
   - SeedEZ SC-C012-0006: add 175 µl
   - SeedEZ SC-C024-0024: add 100 µl
   - SeedEZ SC-C048-0024: add 50 µl
   - SeedEZ SC-C096-0024: add 20 µl

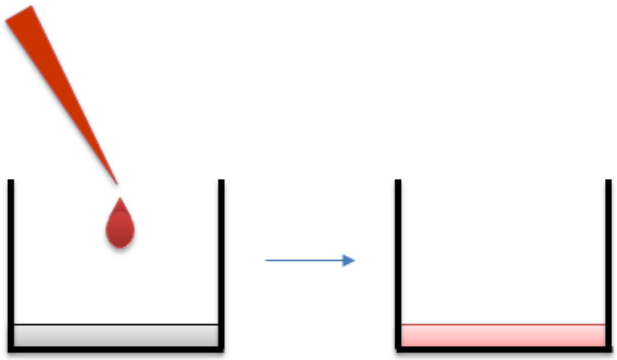
B. Touch SeedEZ surface using micropipette and aspirate the same volume that was dispensed in A (the scaffold will turn white). If the scaffold does not turn white, there is still water in it. Press the SeedEZ surface again and aspirate.

If the coating in Step 3 is very hydrophobic and DI water does not permeate the scaffold uniformly, dip the scaffold into DI water in a 5 ml centrifuge tube.

Note for coated scaffolds:

Some coatings are more or less hydrophobic and may hinder the delivery and distribution of cells within the interior of the scaffold. This step renders the scaffold more "hydrophilic" even under these conditions, and ensures more uniform cell delivery through the scaffold thickness at the time of cell seeding.

Step 5: Add cells to SeedEZ scaffold using micropipette



If you seed cells into **uncoated or coated scaffolds** add:

- SeedEZ SC-C006-0006: 360 µl
- SeedEZ SC-C012-0006: 140 µl
- SeedEZ SC-C024-0024: 80 µl
- SeedEZ SC-C048-0024: 40 µl
- SeedEZ SC-C096-0024: 10-15 µl

After seeding, allow 5 minutes for cells to settle and adhere, and then **slowly** add medium to avoid dislodging cells (see Step 6).

Note:
Although the above volumes may not entirely fill the scaffolds, they are recommended to prevent cells from exiting the scaffold and attaching to the bottom of the plate. If you notice that cells come out, deliver the same number of cells in an even smaller volume next time you seed them.

If you seed cells in a **hydrogel** or an **extracellular matrix** solution add:

- SeedEZ SC-C006-0006: 360-450 µl
- SeedEZ SC-C012-0006: 140-175 µl
- SeedEZ SC-C024-0024: 80-100 µl

- SeedEZ SC-C048-0024: 40-50 µl
- SeedEZ SC-C096-0024: 10-15 µl

After seeding, allow some time for the hydrogel to gel, by following recommendations from the hydrogel supplier, and then add medium (see Step 6).

If you use **multiple cell types**:

- Mix the cells and dispense into SeedEZ, or
- Dispense one cell type, and then another cell type.

Recommended cell seeding densities:

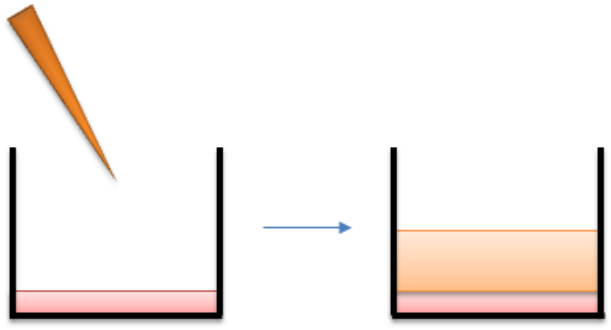
- SeedEZ SC-C006-0006: $9.0 \times 10^5 – 9.0 \times 10^6$ cells
- SeedEZ SC-C012-0006: $3.5 \times 10^5 – 3.5 \times 10^6$ cells
- SeedEZ SC-C024-0024: $2.0 \times 10^5 – 2.0 \times 10^6$ cells
- SeedEZ SC-C048-0024: $1.0 \times 10^5 – 1.0 \times 10^6$ cells
- SeedEZ SC-C096-0024: $0.3 \times 10^5 – 1.5 \times 10^5$ cells

The recommendation is based on the average cell yield ($1 – 1.25 \times 10^5$ cells/cm^2) in confluent tissue culture disposables.

Step 6: Add cell culture medium

Recommended volumes:

SeedEZ SC-C006-0006 in a 6-well plate:	2.00 ml	range 1.900 – 2.900 ml
SeedEZ SC-C012-0006 in a 12-well plate:	1.00 ml	range 0.760 – 1.140 ml
SeedEZ SC-C024-0024 in a 24-well plate:	0.50 ml	range 0.380 – 0.580 ml
SeedEZ SC-C048-0024 in a 48-well plate:	0.25 ml	range 0.190 – 0.285 ml
SeedEZ SC-C096-0024 in a 96-well plate:	0.15-0.20 ml	range 0.100 – 0.200 ml



Step 7: Place the multiwell plate in a humidified, 5% CO_2 incubator.

Note:

The scaffold is approximately 0.5 mm thick. It allows unrestricted cell growth for weeks.

Step 8: Change culture medium

If you culture less than 2 days

- Skip this step.

If you culture longer than 2-3 days

- Exchange medium as in a standard multiwell plates of the corresponding well size.

Recommendation:

Avoid touching SeedEZ culture with a micropipette tip during media changes. To remove culture medium, tilt the plate and aspirate medium.

CELL ISOLATION, QUALITATIVE AND QUANTITATIVE READOUTS

Cell-based assays

Continue with protocols used for multiwell plates of the corresponding well size. SeedEZ is compatible with most cell-based assays, reagents and cell stains.

Recommended assays:

- CellTiter-Blue or alamarBlue for cell viability, proliferation and respiratory metabolism.
- Calcein AM/ Propidium Iodide for live/dead assays.
- CellTiter-Glo, or lysed-LDH assay for cell count.
- LDH release for cell death between feedings or cytotoxicity.
- P450-Glo for cytochrome P450 activity etc.

Plate reader and spectrophotometer readouts

Continue with protocols used for the multiwell plates. Transfer supernatant into another plate or cuvette and read.

Microscopic imaging

Fluorescence imaging is recommended. Stain cells using fluorescent stains you normally use. For example, Calcein AM/ Propidium Iodide for live/dead imaging.

For low-resolution microscopes, image half-through the scaffold thickness, and then flip the scaffold upside down to image the other half.

Cell isolation

See cell recovery protocol online: https://www.lenabio.com/sz_cell_recovery_protocol.pdf

Cell recovery from uncoated scaffolds: wash with 1X Calcium and Magnesium-free PBS first. Use 0.05% to 0.25% Trypsin-EDTA next, if needed.
Cell recovery from coated scaffolds: page 8 in the online protocol.
Cell recovery from hydrogel: page 14 in the online protocol.

Notes:
If cells secrete or are seeded in the extracellular matrix, use suitable enzyme(s) to digest that matrix.
Use sterile forceps to remove SeedEZ scaffold from the plate and during transfer.

Immunocytochemistry

Follow protocols you normally use. Sectioning is not necessary. For high resolution images, use confocal microscopy.
Use sterile forceps to remove the scaffold from the plate and in handling.

Sectioning

Use cryostat. Purchase blades that can cut through glass. Use sterile forceps to remove the scaffold from the plate. Recommended slice thickness is 100 – 200 µm.